

Research Article

Morphological identification and phylogenetic analysis of Asteroidea in the northern coast of the Persian Gulf

Adeli B.¹; Ghavam Mostafavi P.^{1*}; Fatemi M.R.¹

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Abstract

Asteroidae, or starfish, is one of the classes of phylum Echinodermata that its phylogenetic relationships have not been studied in the Persian Gulf so far. In this research, the morphological identification, molecular analysis and phylogeny of 40 asteroid samples were studied from the northern islands of the Persian Gulf. For this purpose, starfish samples were identified using morphological characteristics. The molecular identification was performed based on two fragments of mitochondrial cytochrome oxidase subunit I (COI) and mt 16S rRNA. The phylogenetic relationships of asteroids were analyzed using maximum likelihood, maximum parsimony and Bayesian methods. Eleven species of starfishes belonging to 6 genera as well as 5 families have been identified including *Luidia hardwicki*, *Astropecten hemprichi*, *Astropecten indicus*, *Astropecten polyacanthus phragmorus*, *Astropecten polyacanthus polyacanthus*, of *Culcita novaeguineae*, *Pentaceraster mammillatus*, *Linckia laevigata*, *Linckia multifora*, *Aquilonastra iranica* and *Aquilonastra watersi*. Since the species of *Linckia laevigata* (orange-green morph) and a specimen of *Astropecten polyacanthus polyacanthus* have been identified based on molecular analysis, this technique can be a useful tool in asteroids along with morphological studies. The obtained interspecies distances in genera *Luidia* and *Astropecten* suggest cryptic speciation might have occurred in these genera. The results of Asteroidae classifications at the genus and family levels indicated phylogenetic tree of mt 16S rRNA gene based on Bayesian method is consistent with previous morphological taxonomic and phylogenetic analyses.

Keywords: mt 16S rRNA, Cytochrome oxidase subunit I (COI), Asteroidae, Persian Gulf

1- Department of Marine Sciences, Faculty of Natural Resources and Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran

*Corresponding author's email: mostafavi_pa@srbiau.ac.ir

Introduction

Asteroidea (starfish or sea stars) is one of the most diverse classes of phylum Echinodermata (Knott and Wray, 2000; Matsubara *et al.*, 2004) comprising about 1900 extant species, 36 families and approximately 370 extant genera (Mah and Blake, 2012). This class includes three superorders of Valvatacea, Forcipulatacea and Spinulosacea. Valvataceans distributed worldwide, especially in the tropical Indo-Pacific region. This superorder has some ecologically important families of asteroids including Astropectinidae and Luidiidae (in order Paxillosida) as well as Oreasteridae, Ophidiasteridae and Asterinidae (in order Valvatida) (Mah and Foltz, 2011).

The family Asterinidae cling to hard substrates with flat ventral surface (Waters *et al.*, 2004a) appearing to be detritivores or omnivores. Asterinids are studied in developmental and larval biology (Mah and Blake, 2012). Oreasteridae and Ophidiasteridae are significant members of the ecological communities of coral reefs (Williams, 2000; Mah and Foltz, 2011). As these species are taken for tourist and aquarium/pet industries and become a subject of concern by conservation biologists (Mah and Foltz, 2011). The Astropectinidae and Luidiidae live in sandy or muddy substrate where they feed on molluscs and other invertebrates (Zulliger and Lessios, 2010; Ning *et al.*, 2013).

Asteroids are important in ecological studies addressing the role of

competition, reproduction (Menge, 1975; Meng *et al.*, 1999) and community structure (Paine, 1969, 1974). Asteroids were also study subjects in marine pollution, toxicological global warming (Danis *et al.*, 2003; Georgiades *et al.*, 2006) and ocean acidification (McClintock *et al.*, 2011; Gooding *et al.*, 2009).

The species of asteroids characterizes by color pattern, the number and length of arms, the number of madreporites, papulae, paxillae (in paxillosidans), actinal plates, form of supero and Infero marginal plates and spines, adambulacral and furrow spines (Clark and Row, 1971). Although species of sea stars typically identify by morphological characteristics, the phenotypic diversity is so high in some species of Asteroidea and taxonomic boundaries are difficult to be determined (William, 2000; Zulliger and Lessios, 2010 and Ning *et al.*, 2013). Some morphological characteristics are similar in species of the same genus, such as the number of madreporites in species of *Linckia* that makes difficult to identify morphologically. The genus *Linckia* distinguishes by cylindrical arms, small oral disc, granules and popular pores on aboral surface (William, 2000). Clark and Row (1971) suggested that body color is a useful tool for identification of *Linckia* species, but there are considerable diversities in color of some species. Therefore, their descriptions cannot help us to identify the species with a variety of phenotype.

Echinoderm larvae are pelagic and identified morphologically to family level (Ward *et al.*, 2008). On the other hand, having a long planktonic larval stage resulted in low species diversity in echinoderms. However, the genus *Astropecten* has approximately 150 described species (Zulliger and Lessios, 2010). Although several taxonomic characters such as paxillae and the superomarginal plates with the number and shape of the superomarginal spines contribute for the identification of species, but there are high phenotypic variability in species of *Astropecten* (Gondim, *et al.*, 2014).

The Asteroids of the Persian Gulf have been studied morphologically in previous studies (Mortensen and Heding, 1940; Clark and Row, 1971; Price, 1981, 1982, 1983; Price and Rezai, 1996 and Pourvali, 2015). Since no comprehensive study has been

performed based on molecular identification concerning to identify the species boundary of the starfishes of this region, molecular and phylogenetic analyses of this class can provide more accurate information related to identifications of Asteroidea in the northern parts of the Persian Gulf.

Materials and methods

Sampling and DNA extraction

Forty four specimens from six genera of Asteroids were collected between May 2017 and September 2018 at 7 locations from the northern islands of the Persian Gulf by scuba diving in the subtidal zones (Fig. 1). The samples were transferred to the laboratory and the significant morphological characteristics were photographed using a stereomicroscope (Leica Ez40) and identified based on described morphological features by Clark and Row (1971).

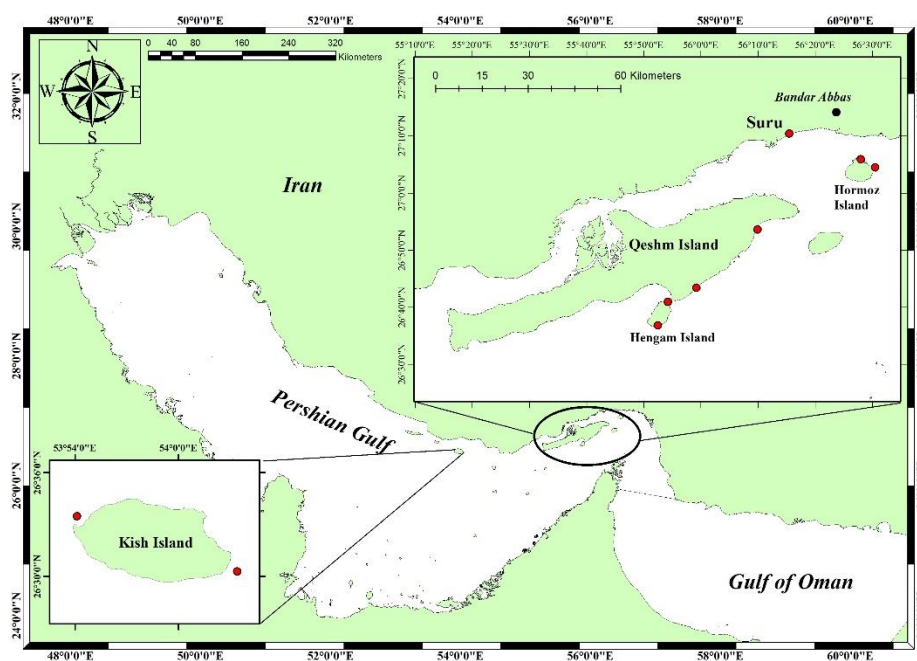


Figure 1: The locations of the sampling stations

61 genomic DNA was extracted from tube foot or arm tip tissue using a 5% Chelex solution (Walsh *et al.*, 1991) or a CTAB-proteinase K extraction buffer (Baker, 1999).

PCR amplification and sequencing

The fragments of the two regions of mitochondrial DNA (mtDNA) were amplified: 1300 bp of the 16S ribosomal RNA (16S) and specific primers of different genera for the cytochrome oxidase sub region I (COI). The primers which were used for DNA

amplification are listed in Table 1. PCR amplification was performed under the following thermal profile: an initial denaturation step at 94°C for 5 min, 30 amplification cycles (94°C for 30 s, 57°C (COI fragment) and 49°C (16S fragment) for 60 s, 72°C for 30 s) and a final extension step at 72°C for 5 min. The amplicons were visualized by 1.5% agarose gel electrophoresis. The PCR products were sent to Bioneer Company in South Korea and sequenced with an ABI- 3730XL analyzer.

Table 1: PCR and the sequencing primers used for the two mitochondrial DNA regions.

Region	Primer	Sequence (5'-3')	Reference
COI	LCOI1490 (F)	GGTCAACAAATCATAAAGATATTG	Folmer <i>et al.</i> , 1994
	HCOI2198 (R)	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> , 1994
	ECOIa(F)	ACCATGCAACTAAGACGATGA	Knott <i>et al.</i> , 2000
	ECOIb(R)	GGTAGTCTGAGTATCGTCGAG	Knott <i>et al.</i> , 2000
	cHCO(F)	TGATTTTTGGTCACCCTGAAGTTT	Folmer <i>et al.</i> , 1994
	EF(F)	ATAATGATAGGAGGRTTTGG	Arndt <i>et al.</i> , 1996
	ER(R)	GCTCGTGTRTCTACRTCCAT	Arndt <i>et al.</i> , 1996
16S	AST16SL2 (F)	CGTAGGATTTTAATGGTGAACAGA	Waters <i>et al.</i> , 2004a
	AST16SR2 (R)	TGTGAAGGAAAGTTGAAATAATGTG	Waters <i>et al.</i> 2004b

* The primers used for amplification of *Astropecten* species.

Molecular identification and phylogenetic analysis

The resulting sequences were blasted in the NCBI database. The nucleotide sequences were deposited in GenBank and their accession numbers were received. The 16S rRNA and COI sequences were aligned by CLUSTAL W software (Thompson *et al.*, 1994). Kimura 2-parameter genetic distances for COI sequences were calculated using MEGA, Ver. 7 (Kumar *et al.*, 2016). Phylogenetic trees were constructed using maximum likelihood

(ML), maximum parsimony (MP), and Bayesian inference (BI) methods.

MP analysis was conducted using PAUP, Ver. 4.0b10 (Swofford, 2002) by heuristic search for 100 random addition replicates using TBR branch swapping and keeping 100 trees per replicate. We assessed clade support in MP analyses performing 1,000 bootstrap resampling replicates. In order to find the best model for ML and Bayesian methods, MrModeltest, Ver. 2.3 (Nylander, 2004) was applied using Akaike information criterion (AIC). The best-fit model was GTR+G+I for

16S data and COI sequences of genus *Astropecten*. The model suggested by MrModeltest was GTR+I for COI data. Constructing ML phylogenetic tree with 1000 bootstrapping was done using MEGA, Ver. 7.

Bayesian analyses were performed in MrBayes 2.3 (Ronquist and Huelsenbeck, 2003). The specification of the program included using a random tree as the MCMC starting point, 25%

as the burn-in proportion, two runs and 500,000 generations to estimate the posterior probabilities of trees.

Two forcipulataceans - *Leptasterias leptodom* (AY548869) and *Asterias forbesi* (AY548858) - were used as an out-group in the phylogenetic tree of 16S gene. In COI tree, *Paracentrotus lividus* (J04815) and *Strongylocentrotus purpuratus* (X12631) were selected as outgroups (Fig. 3).

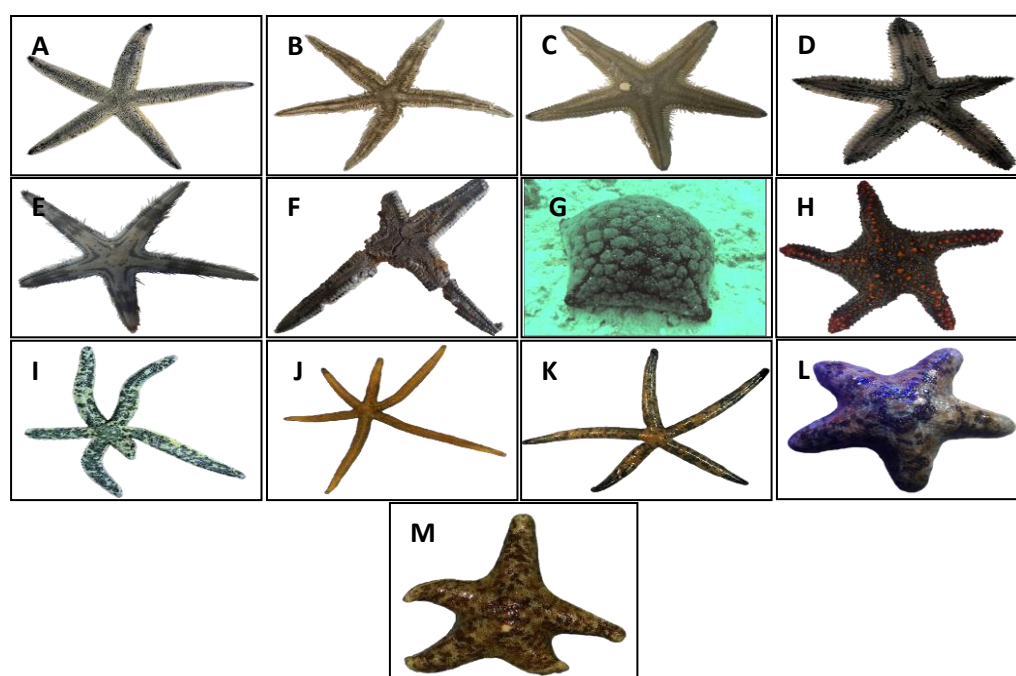


Figure 2: The images of the Asteroids specimens collected in this study. A, *Luidia hardwicki*. B, *Astropecten hemprichi*. C, *Astropecten indicus*. D, *Astropecten polyacanthus phragmorous*. E, *Astropecten polyacanthus polyacanthus*. F, *Astropecten* sp.. G, *Culcita novaeguineae*. H, *Pentacaster mammillatus*. I, *Linckia laevigata*. J, *Linckia multifora*. K, *Linckia* sp.. L, *Aquilonastra iranica*. M, *Aquilonastra watersi*.

Results

Morphological observations

The names of 44 specimens belonging to 10 species and 1 subspecies of Asteroids with summarized morphological characteristics are shown in Table 2. There are many morphological explanations for identification of the species of

Asteroidea. Therefore, distinctive features of each species are mentioned here. Three species of *Astropecten hemprichi*, *Culcita novaeguineae* and *Linckia multifora* were distinguished only based on morphological characteristics, so they have no specimens names.

Table 2: Summary of the morphological characteristics with the specimens' names and identified species.

Specimen names	Morphological characteristics	Identified Species
C4/C6/L1B	R/r= 70/13 mm/The color in live specimens on center disk and carinal lines were darker than the other areas/Bivalved pedicellariae on the outer part of some adambulacral plates/Length of lateral inferomarginal spines equal to the length of two consecutive inferomarginal plates	<i>Luidia hardwicki</i>
-	R/r=40/12mm/Regular arranged Paxillae/inner superomarginal spine and outer superomarginal spine on superomarginal plates/Adambulacral plates had two series of spines with same length and width.	<i>Astropecten hemprichi</i>
1CIN/2CIN/3CIN A12/CA2	R/r=40/12mm/Regular arranged Paxillae/inner superomarginal spine and outer superomarginal spine on superomarginal plates/Adambulacral plates had two series of spines with same length and width.	<i>Astropecten indicus</i>
3CPOL/4CPOL	R/r=41/10/The color pattern is light brown with dark spot/These species had one row consecutive supero marginal spine without any gap.	<i>Astropecten polyacanthus phragmorous</i>
1CPOL/2CPOL	R/r=45/10 mm/The supero marginal spines were reduced after the ones on proximal supero marginal plates/Adambulacral spines were larger than the furrow spines and middle furrow spines were larger than others	<i>Astropecten polyacanthus polyacanthus</i>
ASTR	4 rays; R/r=80/15 mm. Adambulacral spines are more than two series.	<i>Astropecten</i> sp.
-	The body is cushion-like without prominent tubercles. Actinal plates have polygonal granules. The color is light greenish black.	<i>Culcita novaeguineae</i>
Pe1/P1F	R/r= 99/50 mm/Dorsal-lateral spines distinctly smaller than the carinal ones/present of 8 furrow spines and 2-3 subambulacral spines on adambulacral plates and 12 furrow spines and 3-4 subambulacral spines on oral plates	<i>Pentaceraster mammillatus</i>
M1/M1G/M2 M3/M4	R/r= 45/5 mm/ Two madreporites. The color form in live species is green-blue	<i>Linckia laevigata</i>
-	R/r= 50/5mm / Two madreporites. The color form in live species is brownish with red spots	<i>Linckia multifora</i>
M5/M6//M7/M8 /M9/M10/M11/M12	R/r= 47/5 mm /Two madreporites The color form in live species is orange-green	<i>Linckia</i> sp.
A1F/Q1F	R/r=20/10/ The color pattern is grey with red and black spots about 12 short, thick and conical spinelets on proximal abactinal plates in small groups/missing secondary plates on proximal abactinal plates/ rare proximal doubly papulate carinal plates	<i>Aquilonastra iranica</i>
q2/q3/q4/q5/q6/q7 q8/q9/q10	R/r= 26/13/The color in live species is grey with red or green spots about 15 small, thin and long spinelets on proximal abactinal plates up to 4 clusters/secondary plates on proximal abactinal plates/6-7 proximal carinal plates with 1 papula	<i>Aquilonastra watersi</i>

* R/r: The distance from center to arm tip to distance of center to the interradial edge.

Sequence divergence

Kimura 2-Parameter distances were calculated for COI sequences of *Luidia* and *Astropecten* species. There are intra-species divergences of 0.8-1.1% and 0.4-12.9% in *Luidia hardwicki* and *Astropecten* individuals from the Persian Gulf, respectively. *Luidia hardwicki* individuals from sea of China

DNA sequence and phylogenetic analysis

Mt 16S rRNA sequences

The mt 16S rRNA sequences of *Aquilonastra watersi*, *Pentaceraster mammillatus* and *Luidia hardwicki* species were first reported in the gene bank. *Aquilonastra watersi* sequence with *Aquilonastra yairi* from Waters *et al.* (2004a) and *Pentaceraster mammillatus* with *Pentaceraster cumingi* from Mah and Foltz (2011) were placed with high support (ML=100%, MP=100%, PP=100%) in a sister taxon. The genus *Aquilonastra* formed a moderately supported monophyletic clade (ML=44%, MP=59%, and PP=78%) with genus *Pentaceraster*.

Luidia hardwicki with strong support (100% in all analysis) was sister to *Luidia quinalia* (D63743) species. *Astropecten indicus* sequence formed highly supported (ML=100%, MP=99%, and PP=100%) sister taxon with *Astropecten indicus* from Zulliger and Lessios, 2010. Genus *Luidia* was grouped with genus *Astropecten* with the posterior probability of 51%.

and Persian Gulf showed genetic distances at the range of 14.9-15.9%. Genetic distances in *Astropecten indicus* individuals from Persian Gulf and Indo-Pacific clade (Zulliger and Lessios, 2010) ranged from 8.9 to 9.4%, and this distance in *Astropecten polyacanthus* sequences from these regions was at the range of 10.6-13.2%.

The groups of *Aquilonastra/Pentaceraster* and *Luidia/Astropecten* formed a monophyletic clade with a high posterior probability 93%.

Linckia laevigata specimens formed a sister taxon with *Linckia laevigata* (AY279318, DQ297095) with a strong bootstrap (ML=100%, MP=100%) and moderate posterior probability of 63%. Genus *Linckia* was clustered with different bootstraps supported (ML=47%, MP=95%) and a strong support in Bayesian inference (100%) with *Aquilonastra/ Pentaceraster /Luidia/ Astropecten* clade. The examined genera belonging to superorder Valvatacea in a monophyletic clade rooted against forcipulatacean sea stars (Fig. 3).

COI sequences

Linckia laevigata specimens formed a monophyletic clade with *Linckia laevigata* from Williams, 2000 (ML=90%, MP=73%, PP=57%). *Luidia hardwicki* sequence placed in a sister group with *Luidia hardwicki* species in the nominal taxa from China Ning *et al.* (2013) (ML=97%, MP=90%, PP=100%). The COI sequences of

Aquilonastra watersi species were first reported in the gene bank and formed a strong supported sister taxon (ML=100%, MP=100%, PP=98%) with *Aquilonastra yairi* (AY370752). Although the specimen of CA2 was amplified with ECOIa and HCO primers, it was first reported to the

genebank (MT375399). The results of COI phylogenetic tree showed different features in genus level with 16S tree, including genera *Linckia* and *Luidia* forming a moderate supported monophyletic clade (ML=41%, MP=50%, PP=52%) (Fig. 4).

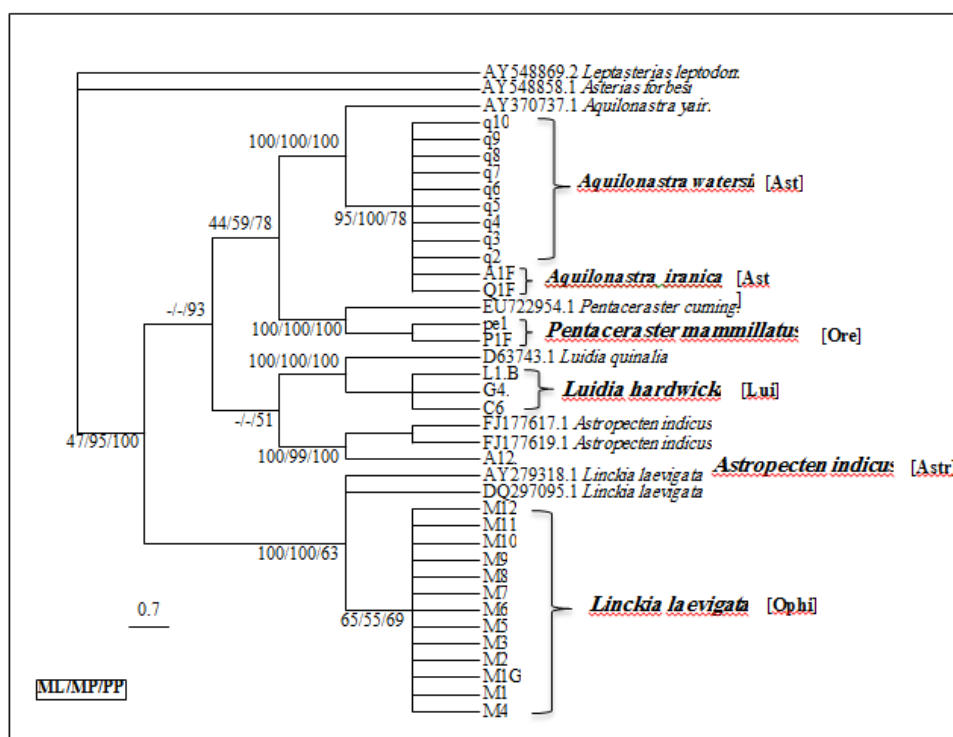


Figure 3: The molecular phylogeny of Asteroids species based on the 16S rRNA sequences. The first numbers are from the maximum likelihood analyses (1000 replications), the second numbers are from the parsimony analyses (1000 replications) and the last numbers are from Bayesian method. For comparison with the traditional taxonomic, the family of each species has been indicated using the following abbreviations: Ast, Asterinidae; Ore, Oreasteridae; Ophi, Ophiasteridae; (Valvatidan) Lui, Luidiidae; Astr, Astropectinidae; (Paxillosidan).

In COI phylogenetic tree of genus *Astropecten* (Fig. 5), *Astropecten indicus* and *Astropecten polyacanthus* sequences were clustered with high support in a sister taxon with *Astropecten indicus* (ML=100%, MP=96%, PP=100%) and *Astropecten polyacanthus* (ML=98%, MP=95%,

PP=100%) reported by Zulliger and Lessios (2010), respectively. *Astropecten indicus* and *Astropecten polyacanthus* species were grouped within a highly supported (MP=100%, PP=100%) monophyletic clade A (Indo Pacific) from Zulliger and Lessios (2010).

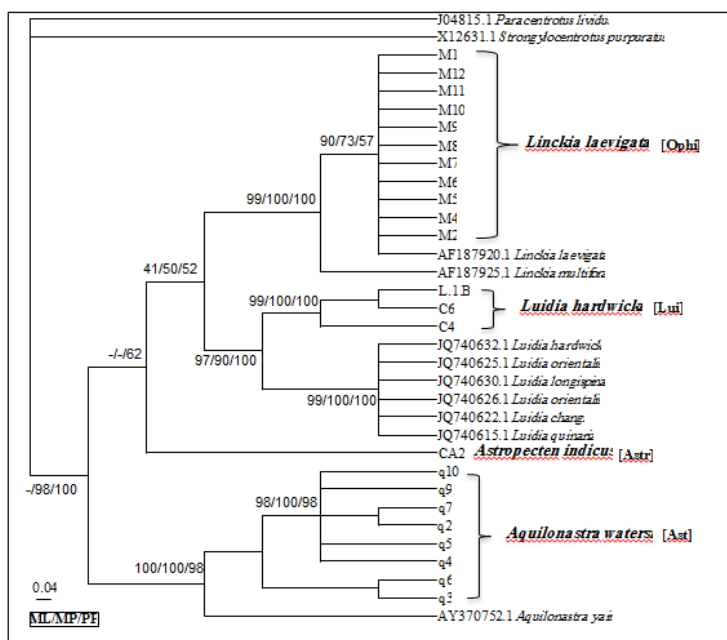


Figure 4: The molecular phylogeny of the Asteroids species based on the COI sequences. Other details are the same as in Figure 3.

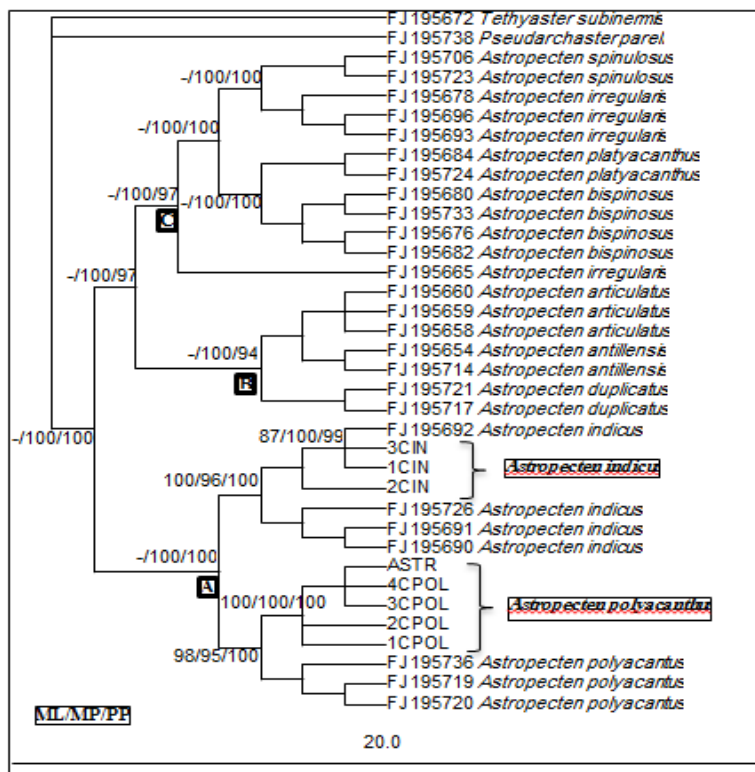


Figure 5: The molecular phylogeny of *Astropecten* species based on the COI sequences. The first numbers are from maximum likelihood analyses (1000 replications), the second numbers are from parsimony analyses (1000 replications) and the last numbers are from Bayesian method. The species of *Astropecten indicus* and *Astropecten polyacanthus* were grouped in monophyletic clade A (Indo-Pacific) (Zulliger and Lessios, 2010) (Clade B: The east and west coasts of America; clade C: Eastern Atlantic and Mediterranean areas).

Discussion

Consistency of the morphological characteristics and molecular identifications

COI as a barcoding marker has been used successfully in many animal taxa (Bingpeng *et al.*, 2018). So, we used COI gene for molecular identifications of Asteroidea specimens at species level.

The species of *Luidia hardwicki* identified based on morphological features. The length of lateral inferomarginal spines was equal to the length of two consecutive inferomarginal plates (Fig. 2A). The results of COI phylogenetic tree showed that the *Luidia hardwicki* sequences were placed in a sister group with nominal species from Ning *et al.* (2013). The genetic distance between *Luidia hardwicki* sequences of Persian Gulf and Sea of China was at the range of 14.9-15.9%. Ning *et al.* (2013) found a genetic divergence existent between *Luidia quinaria* individuals from the Sea of Japan and Sea of China (12.9-13.1%). Given that this distance is greater than the intra-specific distance proposed by Ward *et al.* (2008) (0-1.85%), Ning *et al.* (2013) suggested that there is a probability of cryptic speciation and morphological descriptions which are required to be reassessed.

Genus *Astropecten* is one of the most complex genera in the class Asteroidea that species shows morphological variability result in different subspecies (Zulliger and Lessios, 2010). Previous studies suggest

that the number and shape of the superomarginal spines are useful for the identification of species but the number and shape of the adambulacral spines proved to be very similar among specimens. So, it cannot be a good character for the identification of species (Gondim *et al.*, 2014). The diagnostic characteristic in two subspecies of *Astropecten polyacanthus polyacanthus* and *Astropecten polyacanthus phragmorus* is present or absent of supero marginal spines (Clark and Row, 1971). The specimens of 1CPOL and 2CPOL sequences (Fig. 2E) of genus *Astropecten* do not have a supero marginal plate spines on some plates. The samples of 3CPOL and 4CPOL sequences (Fig. 2D) have supero marginal plate spines on all the plates. The sequences of these two species were grouped in a monophyletic clade with one different nucleotide. The sample of ASTR sequence (Fig. 2F) have 4 arms and the ratio of R/r=80/15. In this specimen, some adambulacral spines were more than two series. So, we thought that it was not *Astropecten polyacanthus*, but the results of molecular identification shown ASTR sequence was grouped with high support with *Astropecten polyacanthus* sequences. We observed that some supero marginal plates do not have spines in ASTR, but placed in 3CPOL and 4CPOL clade (*Astropecten polyacanthus phragmorus*). Mortensen and Heding (1940) found some specimens of *Astropecten* from the Persian Gulf which had an intermediate

form of supero marginal spines between *A. polyacanthus* and *A. phragmorus*.

Astropecten polyacanthus from the Persian Gulf indicated genetic diversity with *Astropecten polyacanthus* from Philippines and Japan which was at the range of 10.6-13.2%. *Astropecten indicus* individuals were genetically distinct from Brunei and Pakistan with genetic distance over 6%. Considering the intra-specific distance 0-1.85% of Asteroidea, *Astropecten polyacanthus* and *Astropecten indicus* species are species-complex (Zulliger and Lessios, 2010) and the cryptic speciation is likely to occur in these groups.

The specimens from genus *Linckia* in the Persian Gulf were observed in three color patterns: Blue with green spots (Fig. 2I), brownish with red spots (Fig. 2J) and the latter orange with green spots (Fig. 2K) in aboral surface. Clark and Rowe (1971) have mentioned that the species of *Linckia* have blue and green-blue morphs and the ratio of R/r is 5-10/1, which is named as *Laevigata*. Also *multifora* was brownish and the ratio of R/r is more than 10/1. Thus, the green-blue and brownish-red specimens were identified. Related to orange-green morph, our molecular results showed that these sequences were clustered in a monophyletic clade with *Linckia laevigata* suggested by Williams (2000) and were sister to *Linckia multifora* (AF187925) sequence in COI phylogenetic tree. So, the identification of *Linckia* species based on molecular data highlights the importance of using molecular analysis.

The COI sequences of two morphs of *Linckia laevigata* did not show genetic distance and there is no difference in their nucleotides. Williams (2000) suggested that the species of *Linckia* were an ecophenotype and the color patterns may be differed by environmental factors (e.g. light intensity or water temperature). In our study, the samples with blue-green morph were common in intertidal zone and the orange-green morph ones were collected in subtidal zone. These results were also presented by Strong (1975).

The specimens of genus *Aquilonastra* were identified morphologically as the species of *Aquilonastra watersi* (Fig. 2L) and *Aquilonastra iranica* (Fig. 2M). The species of *Aquilonastra iranica* differed in number and form of aboral surface spinelets from *Aquilonastra watersi*. There are also no secondary plates in proximal abactinal plates but the results of the molecular identification based on 16S gene did not indicate any differences in sequences of two species. Our experiments were not successful in sequencing of specimens A1F and Q1F (*Aquilonastra iranica*) for COI gene.

Phylogenetic relationships

Although the Asteroids have low species diversity in the Persian Gulf and it is not possible to discuss the details of their phylogeny relationships, while two subjects have always been discussed in phylogenetic tree of Asteroids that can be seen in our results. The first subject is determining the root of the Asteroid

tree. When the tree is rooted against the other echinoderm sequences, one of the taxa of Paxillosida or Valvatida put close to root. According to proposed root of Mah and Foltz (2011), when Valvataceans is rooted against superorder Forcipulatacea, the taxonomic relationships amongst taxa are similar to the phylogenetic classification of the Valvatacea as proposed by Blake (1987). Mah and Foltz (2011) presented the most comprehensively sampled molecular phylogeny yet produced for the Valvatacea. The second case is phylogenetic position of Paxillosidans. Although many studies had not supported a monophyletic Paxillosida (e.g. Wada *et al.*, 1996; Knott and Wray, 2000; Matsubara *et al.*, 2004), Paxillosidans (Luidiidae and Astropectiniidae) formed a monophyletic clade in the results of Bayesian analysis of 16S tree.

The phylogenetic relationships can be resolved with relatively fast-evolving sequences such as mt 16S rRNA at the genus and family level (Mah and Foltz, 2011). Our results for 16S phylogenetic tree indicated similarities to phylogenetic tree according to Mah and Foltz (2011). The families of Asterinidae and Oreasteridae (Valvatida) were grouped in a monophyletic clade. The family of Ophidiasteridae (valvatidan) with cylindrical arms and small disk was placed in a distinct clade from the other valvatidans. The results of COI phylogeny analyses were not consistent

with family relationships among taxa in 16S tree.

Considering the intraspecies and interspecies divergence of Asteroidea in COI gene ranged from 0 to 1.85% and 2.17 to 22.85% respectively, occasionally morphologically different species are genetically indistinguishable from each other like *Linckia laevigata* and *Aquilonastra* species. Morphological differences may be arising from environmental variations in these species. In contrast, some species with genetic divergence have similar morphological characteristics such as genus *Astropecten* and *Luidia* and cryptic speciation may be occurred. Morphological features should be more investigated in these species. Our results show that the combined analyses based on molecular sequencing and morphological evidence represents a useful technique for Asteroid taxonomy.

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